SITE DIRECTED MUTAGENSIS OF OUTER MEMBRANE CYTOCHROMES IN *SHEWANELLA ONEIDENSIS* MR-*1*,

A.O. Szadkowski[1], C.R. Myers*[2], St. Norbert College[1], Department of Natural Sciences, De Pere, WI 54115, Medical College of Wisconsin[2], Department of Pharmacology and Toxicology, Milwaukee, WI 53226, adam.szadkowski@snc.edu.

Shewanella oneidensis is a metal reducing bacterium that contains outer membrane cytochromes. These *c* type cytochromes contain 10 heme groups and can transport electrons across the outer membrane to reduce extracellular insoluble metals such as manganese or iron. It is hypothesized that the electrons may travel through the cytochrome by traveling sequentially through each heme (electric wire hypothesis), or there could be multiple heme transfer clusters (1-5, 6-10). To test the electric wire hypothesis, each of the heme groups will be mutated individually to create nonaheme variants. This study focused on the site directed mutagenesis of heme groups 1-4. By using a mutagenesis kit and specific primers a previously made heme four mutation was constructed, but attempts to incorporate this mutation into the wild type by a double crossover were ineffective. Further tests demonstrated that the bacteria had single crossovers, but it would cross the mutation vector back out, suggesting that the flanking regions might be too long. Mutations were produced for hemes two and three, although heme two had multiple insertions. Further study indicates that the mutations for hemes one and two might be successful if longer primers are used. The heme three mutations were successfully ligated into a GFP expression vector and electroporated into *E. coli*. Currently we are testing the heme three construct to see if its shorter flanking regions will increase our probability for a double crossover.